UPTAKE OF ORGANIC MATERIAL BY AQUATIC INVERTEBRATES. III. UPTAKE OF GLYCINE BY BRACKISH-WATER ANNELIDS[1]

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In previous work (Stephens and Schinske, 1961; Stephens, 1962a; Stephens, in press) it has been shown that a number of marine invertebrates are capable of removing glucose and amino acids from dilute solution in the surrounding sea water. In the case of the maldanid worm, *Clymenella torquata* (Stephens, 1962b, 1962c), measurements were made of the rate of uptake of amino acids and compared with the level at which these compounds occurred in the habitat of the organism. These results suggested that this uptake process might contribute significantly to the nutrition of these animals.

Repeated attempts have been made in this laboratory to demonstrate uptake of amino acids and sugars in fresh-water invertebrate Metazoa. Twelve genera, representing six phyla (Table I), were examined with respect to their capacity to

TABLE I

Fresh-water organisms examined for their ability to remove glucose and glycine from solution

Phylum Porifera
Spongilla lacustris

Phylum Cnidaria
Hydra oligactis

Phylum Platyhelminthes
Dugesia dorotocephala

Phylum Ectoprocta
Pectinatella magnifica

Phylum Annelida
Lumbricus terrestris
Placobdella parasitica

Phylum Mollusca
Physa sp.
Limnaea stagnalis
Sphaerium sp.
Pisidium sp.
Amblema costata
Eliptio dilatatus

remove glucose and glycine from dilute solution, using C^{14}-labelled compounds. In no case could removal of more than a few per cent of these compounds in a 24-hour period be demonstrated. In a few cases where the labelled carbon was observed to decrease more rapidly in the ambient medium, addition of neomycin reduced the apparent rate of disappearance by 80% to 90%. Although it certainly cannot be maintained that any of the fresh-water forms employed are closely related to marine invertebrates previously studied, these uniformly negative results are in sharp contrast to the report of Stephens and Schinske (1961). They observed uptake of glycine by all of the genera of marine invertebrates employed (except arthropods), regardless of taxonomic position, habitat, and predominant feeding mechanisms.

It was then attractive to examine an organism capable of tolerating extreme changes in salinity, in the hope of establishing some relationship between the

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osmotic concentration of the medium and the capacity for uptake of small organic molecules. The brackish-water nereids offer a number of advantages for such an investigation. They are capable of surviving abrupt transfers within a very broad range of salinities. These worms have served as the subject of a number of studies which have provided information concerning their distribution, their capacity for osmoregulation and for chloride regulation, and other features of their physiological ecology. A summary and review of some of this information is presented by Smith (1959a). The fact that data in the literature permit an estimate to be made of the salinity at which osmoregulation and chloride regulation become conspicuous is particularly desirable. This permits a more searching exploration of the potential relationship between uptake of small organic compounds and such processes. The present report presents the results of work carried out to investigate such a relationship in two species of nereids.

Material and Methods

Two species of *Nereis* (*sensu lato*) were employed. *Nereis limnicola* Johnson (=*Neathes lighti* Hartman: see Smith, 1959b, concerning synonymy) was collected from Lake Merced and Walker Creek in California. The chloride content of Lake Merced was measured as 3.18 meq./liter which is a salinity of approximately 0.216‰. This is then a fresh-water habitat according to the Venice System of classification (Caspers, 1959). At the time of collection at Walker Creek, the chloride content of the surface water was 72 meq./liter and the interstitial water was 123 meq./liter, a mixohaline environment in an estuarine situation where considerable fluctuations would be expected due to the tidal cycle.

The second species employed was *Nereis succinea* Frey and Leuckart (= *Neathes succinea*).[2] Collections were made in San Francisco Bay, at chloride concentrations of 285 to 405 meq./liter (approximate salinities, 18.00‰ to 25.50‰), the mixopolyhaline zone in the Venice System.

Animals were collected as required and maintained in the laboratory in large fingerbowls. About a centimeter of sand from the collection site was provided as a substrate covered by one to two centimeters of sea water or sea water diluted with pond water to provide lower salinities. Animals which did not burrow in the substrate were not used nor were animals used within 48 hours of collection. The exception to this last statement is the set of observations dealing with the change in rate of uptake with time where measurements were made 24 hours after collection of *Nereis limnicola*. Animals subjected to changes in salinity in the laboratory were allowed at least 48 hours to accommodate to the change.

In determining the uptake of glycine, the following procedure was used. Dilutions of sea water were prepared, using glass-distilled water, to give the same chloride concentration as the medium in which the animals were being maintained. Measured amounts of glycine-2-C^{14} were added to this diluted sea water. Groups of animals were then placed in a suitable volume of this solution for 15 minutes to one hour. After this exposure, the animals were rinsed in diluted sea water of the same chloride content and weighed to the nearest milligram. They were then

[2] *Nereis succinea* Frey and Leuckart is considered conspecific with *Nereis limbata* Ehlers of the Woods Hole region.

extracted at room temperature in 2.0 ml. of 80% ethanol (5.0 ml. in some cases) for 24 hours. Radioactivity of the alcohol extract was determined by evaporating duplicate 0.5-ml. samples on planchets and counting with a thin window Geiger-Muller detector. In some cases, the animals were extracted for an additional 24 hours with 5 to 10 ml. of 80% ethanol, breis prepared in a measured volume of distilled water, using a tissue homogenizer, and the radioactivity of the alcohol-insoluble fraction determined. Initial and final measurements were made of the radioactivity of the medium. In all cases, the data have been corrected for background and sample thickness.

Chloride concentrations were measured electrometrically with a chloridometer in samples taken at the time of collections. Media of chloride content lower than sea water were prepared volumetrically from sea water of known chlorosity.

Results

Observations on *Nereis limnicola* will be reported first and *Nereis succinea* introduced later for comparative purposes.

Twenty-two *N. limnicola* collected at Lake Merced were acclimated for four days to sea water at a concentration of 543 meq. Cl^-/liter. They were then placed in 100 ml. of sea water at this concentration containing 0.15 mg. glycine-2-C^{14} (2×10^{-5} *M*/l., 400 cpm.). After one hour the animals were rinsed in sea water, weighed, and placed individually in 2.0 ml. of 80% ethanol. The radioactivity (2000 counts, background approximately 19 cpm) in the alcohol extract was measured 24 hours later. A regression line was calculated for the log of cpm in 0.5 ml. of the alcohol extract (this ranged from 52 to 282 cpm in these observations) as a function of the log of wet weight. The slope obtained was 0.483 ± 0.052. By making suitable corrections for the absorption of the samples, the ratio of alcohol-soluble radioactivity per unit volume to ambient radioactivity could be calculated. This accumulation ratio ranged from 0.75 to 2.72 and was negatively correlated with weight, as would be expected on the basis of the regression line reported above. An alternative way of expressing the rate of uptake for such a group of animals is used in much of the data to be reported. The radioactivity of the alcohol extract is divided by the square root of the wet weight (as an acceptable and convenient approximation of the exponent, 0.483, relating uptake and weight). Uptake can then be calculated for a selected weight. The animals employed in these observations ranged in weight from 56 to 472 mg. and averaged approximately 200 mg. The arbitrary factor representing uptake for this group is 7.88 ± 2.35. Suitable calculations indicate an accumulation ratio of 1.75 for a 200-mg. animal. Alternately, one may calculate that an average 200-mg. animal acquired 0.53 ± 0.16 micrograms of glycine which remained in alcohol-soluble form. The justification for referring to the ratio of alcohol-soluble radioactivity to ambient activity as an accumulation ratio comes from chromatography of the alcohol-soluble material. The radioactivity resides in glycine in the extract.

In another group of animals, individuals were removed at 30 minutes and at 60 minutes. Radioactivity of the extract divided by the square root of wet weight was, respectively, 22.7 ± 5.9 ($n = 9$) and 45.5 ± 17.6 ($n = 9$). Hence the process is linear with time for at least 60 minutes.

Groups of animals were tested for their capacity to remove glycine from

ambient sea water at various chlorosities. The worms were tested three or four days after they were placed in water of the designated concentration. In order to compensate for differences in the ambient glycine-2-C^{14}, which ranged from 10^{-5} to 3×10^{-5} M/l., uptake is expressed in arbitrary units derived as follows. The

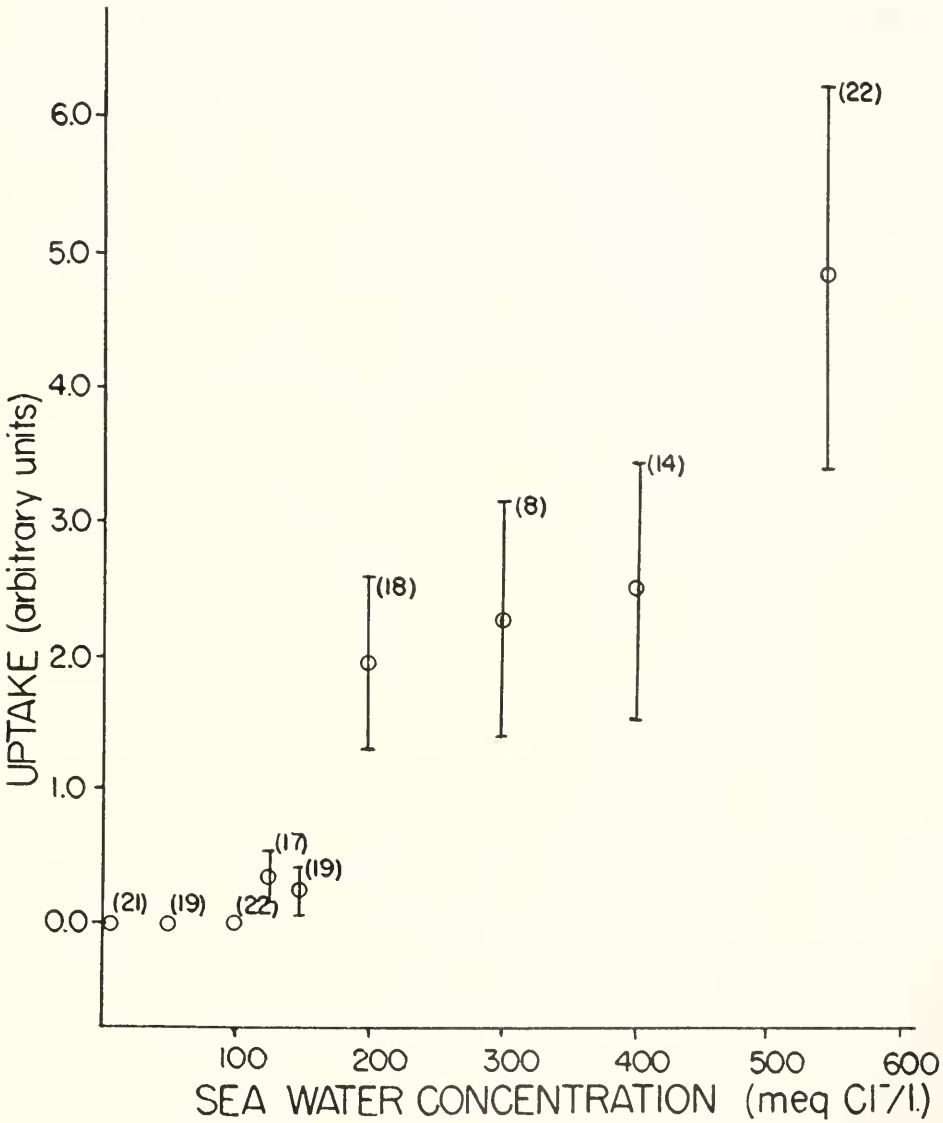

FIGURE 1. Rate of uptake of glycine by *Nereis limnicola* as a function of the chlorosity of the ambient medium. The bars represent standard deviations, and the numbers in parentheses are the number of individuals observed. Uptake is in arbitrary units (see text) and has been corrected for the weight of the animals and for differences in ambient radioactivity of the solutions.

radioactivity of 0.5 ml. of the ethanol extract was divided by the square root of the wet weight and this was in turn divided by the initial ambient radioactivity. The result was multiplied by 1000. Figure 1 presents the results of such observations. Standard deviations are not presented for sea water concentrations of 3, 50 and 100 meq. Cl^-/l. since none of the radioactivities of the alcohol extracts was significantly different from background. After an additional extraction with 80% alcohol, breis of the animals were prepared and alcohol-insoluble radioactivity determined. Values ranged from 48% to 71% of the alcohol-soluble radioactivity and were not related to ambient concentration of sea water. No significant alcohol-insoluble activity could be demonstrated below 125 meq. Cl^-/l.

In the course of these observations, a group of animals which had been maintained in sea water at a concentration of 300 meq. Cl^-/l. for a period of 63 days was employed. These animals exhibited a rate of uptake which was 9.7 times greater than that presented in Figure 1 at this salinity. This suggested that the adaptation of this process to salinity change might occur much more slowly than

TABLE II

Mean uptake and standard deviation for groups of animals maintained at 300 meq Cl^-/l. for the time listed. Data have been corrected for variation in weight and ambient radioactivity. Ambient glycine concentrations range from 1 to 3 $\times$ 10^{-5} molar

Days	Uptake*
Animals collected at Lake Merced	
1	1.74 ± 0.93 ($n = 12$)
4	3.16 ± 0.77 ($n = 12$)
7	6.87 ± 3.90 ($n = 12$)
11	14.55 ± 5.06 ($n = 11$)
14	17.75 ± 9.25 ($n = 12$)
Animals collected at Walker Creek	
1	7.84 ± 3.13 ($n = 12$)
4	14.70 ± 5.21 ($n = 12$)

* Uptake is expressed in arbitrary units. See text.

had been assumed. To test this, worms were collected from Lake Merced and placed in sea water at 300 meq. Cl^-/l., and their capacity for glycine uptake checked periodically. Table II presents uptake in arbitrary units (corrected for weight and ambient concentration) at various times after the animals were placed at this concentration. It is clear that an approximately ten-fold increase in the rate of glycine uptake has occurred during the two weeks the process was followed, and that the adaptive capacity of the system had not necessarily been exhausted at the end of this period.

It was desirable to look at rates of glycine uptake in worms collected at Walker Creek since they represent an estuarine population and might be expected to be exposed to higher salinities than would be the case in the fresh-water environment of Lake Merced. As indicated in Table II, the Walker Creek animals did indeed exhibit a higher initial rate of uptake at the test salinity. They also showed an increase in rate after acclimating for four days. Unfortunately, neither population could be followed beyond the times indicated in Table II. It is perhaps worthy of

comment that a population of worms kept at 50 meq. Cl^-/l. for three weeks showed no demonstrable uptake of glycine at the end of this period.

It was of interest to examine the relationship between ambient glycine concentration and the rate of uptake. Concentration curves were determined for groups of animals at sea water concentrations of 200, 300, and 520 meq. Cl^-/l. The animals in the first and third groups were collected at Lake Merced while the animals measured at 300 meq. were collected from Walker Creek. They had been maintained in the laboratory at the chlorosities indicated for 4, 4, and 6 days, respectively. Glycine concentrations ranged from 2×10^{-5} to 10^{-3} moles per liter for each group. A plot of the reciprocal of ambient glycine concentration against the reciprocal of rate of uptake gave a reasonable fit to a straight line. Correlation coefficients for the least squares regression lines were 0.74 ($n = 22$), 0.62 ($n = 36$), and 0.67 ($n = 23$) for the three groups. This procedure is equivalent to a Burk-Lineweaver plot in enzyme kinetics. Obtaining a straight line suggests that an adsorptive step becomes rate-limiting as ambient glycine concentration is increased. By analogy with an enzyme-catalyzed reaction, it is possible to calculate the apparent maximum velocity of uptake (V_{max}) from the intercept of such a plot and to calculate the concentration at which velocity is half maximal (K_m) from the intercept and the

TABLE III

V_{max} and K_m values with standard deviations for three groups of Nereis limnicola. *The groups are identified by the sea water concentration at which they were maintained, expressed as meq. Cl^-/l., and by their place of collection. Units for V_{max} are moles $\times 10^{-9}$ per 200-mg. worm per hour. Units for K_m are moles $\times 10^{-5}$ glycine per liter*

Group	V_{max}	K_m
200 meq. (Merced)	4.07 ± 1.12	15.0 ± 3.0
300 meq. (Walker)	22.7 ± 2.5	3.8 ± 0.9
520 meq. (Merced)	24.7 ± 6.9	3.7 ± 1.9

slope of the regression line. These figures and their standard deviations are listed in Table III. The V_{max} for the 200-meq. group is significantly below that for the other groups, which do not differ significantly. The K_m for the 200-meq. group is also significantly higher than that for the other groups. The data have been corrected for the size of the worms and rates are expressed on the basis of averages for 200-mg. animals. A detailed interpretation of the apparent differences would require postulating a mechanism for uptake which would permit identification of the analogues corresponding to changes in enzyme concentration, turnover number, and the complex of rate constants expressed in the K_m. This does not seem justifiable. Hence, it can simply be pointed out that the differences in rate which are apparent at low ambient concentrations are also manifest as differences in maximum velocity. The data indicate that this change is not produced merely by an increase in the number of uptake sites available since this would not modify the K_m.

The facts that the relation between weight and uptake is an exponential one, and that the rate of uptake varies with ambient glycine concentration in the fashion described, suggest that uptake might be occurring directly across the surface of the animals without the necessary involvement of the gut. More direct evidence can be presented for this position. A group of animals ($n = 9$) ligated with thread at

approximately the tenth setiger was compared with a control group ($n = 9$). Uptake of glycine in the two groups in arbitrary units was 10.69 ± 4.85 and 11.84 ± 4.54, respectively. When a group of worms ($n = 8$) which had been decapitated 24 hours before testing was compared with a control group ($n = 12$), uptake was 4.02 ± 1.01 and 4.17 ± 1.01, respectively. The control groups are not comparable. These procedures do not exclude the possibility of uptake *via* the posterior gut but serve to strengthen the suggestion that uptake occurs across the body wall, as

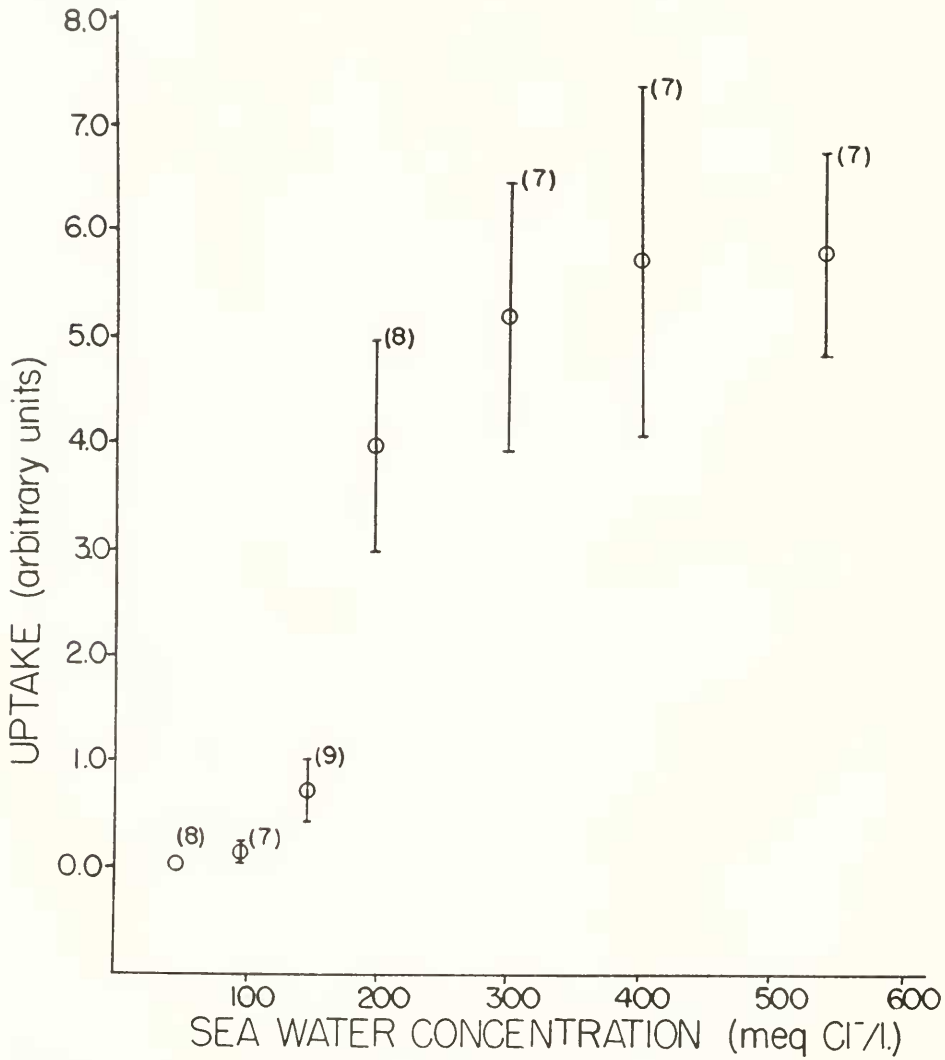

FIGURE 2. Rate of uptake of glycine by *Nereis succinea* as a function of the chlorosity of the ambient medium. The bars represent standard deviations, and the number of individuals observed is indicated in the parentheses. Uptake has been corrected for weight and for differences in ambient radioactivity.

has been reported for the maldanid annelid, *Clymenella torquata* (Stephens, in press).

Comparable observations were made on groups of *Nereis succinea*. The data present some striking similarities but also indicate significant differences between the two species. Figure 2 indicates the results of observations testing the relation between sea water concentration and the rate of uptake. The arbitrary units in which uptake is expressed are not the same as those presented for *Nereis limnicola*. In fact the rate of uptake for *N. succinea* is strikingly greater. After 15 minutes' exposure to an ambient glycine concentration of 2.7×10^{-5} *M*/l. in sea water at 520 meq. Cl^-/l., the ratio of alcohol-soluble radioactivity in the body water of the worms to the ambient radioactivity was 9.8 ± 2.4. This is the mean figure for a 200-mg. worm and is comparable to the data for *N. limnicola*. Thus, accumulation is proceeding at approximately 22 times the rate observed for *N. limnicola*. Another distinction between the animals is the absence of any indication of an influence of chloride concentration on uptake of glycine above 200 meq. Cl^-/l.

TABLE IV

Mean uptake and standard deviation for groups exposed to glycine at approximately 2×10^{-5} moles per liter in solutions of the compounds listed. All solutions are approximately equivalent in osmotic concentration to sea water at 200 meq. Cl^-/l.

Solution	Uptake*	% uptake in sea water
Nereis succinea		
sea water	180 ± 42	100%
NaCl	122 ± 32	68%
galactose	30 ± 7	17%
Nereis limnicola		
sea water	5.9 ± 2.8	100%
NaCl	2.6 ± 0.9	44%
galactose	1.1 ± 0.6	19%

* Uptake is expressed in the same arbitrary units used in Table II.

The greater rate of glycine uptake exhibited by *N. succinea* is also apparent in the maximum velocity of uptake estimated from a Burk-Lineweaver plot of data relating ambient concentration and uptake. As in the case of *N. limnicola*, a straight line is obtained from such a plot. The maximum velocity calculated from the regression line at 200 meq. Cl^-/liter is $287 \pm 59 \times 10^{-9}$ moles per 200-mg. worm per hour. The K_m is $2.24 \pm 0.94 \times 10^{-4}$ moles glycine per liter. Comparable figures at 520 meq. Cl^-/l. are $322 \pm 59 \times 10^{-9}$ moles per worm per hour with a K_m of $1.13 \pm 0.47 \times 10^{-4}$ moles glycine per liter. The differences in the two groups are not significant.

It is apparent from Figures 1 and 2 that in the case of both animals, a sharp decrease in rate of uptake of glycine occurs between 200 and 150 meq. Cl^-/l. and that uptake has entirely or virtually stopped at 100 meq. A series of observations was undertaken to determine whether glycine uptake would proceed in other solutions whose osmotic concentration was approximately that of sea water at 200 meq. Cl^-/l. Solutions of galactose (400 m*M*/l.) and sodium chloride (200 m*M*/l.) were employed. Galactose was used since *N. limnicola* was observed to take up glucose

from dilute solutions. Although galactose was not directly checked as a possible substrate for uptake, previous work (Stephens, 1962a, and unpublished observations) indicated that galactose is not removed from solution by the coral, *Fungia,* or the clam, *Mercenaria mercenaria.* This of course does not exclude the possibility that it may be taken up by nereids. Table IV presents uptake in arbitrary units from the ambient solutions listed. In each case, glycine concentration was approximately 2×10^{-5} *M*/l. Worms of both species survived such exposures but were clearly not normal in their behavior. The worms in galactose became quite sluggish, while those in sodium chloride collected in a tangled mass in a fashion which never occurred at any concentration of sea water. Hence, the decrease in rate of uptake in galactose and sodium chloride cannot be referred to specific ion effects with any confidence, since toxic effects of the solutions are probable. One may conclude that provided the osmotic concentration of the medium is raised to this level, at least some uptake occurs.

Discussion

Both species of nereids employed in these observations are capable of removing glycine from dilute solution in the ambient medium at intermediate and high salinities. Both species fail to exhibit this capacity at sea water concentrations below 50 meq. Cl^-/l. In both cases, a dramatic reduction in rate of uptake occurs between 200 and 150 meq. Cl^-/l. It is of interest to examine the available data concerning osmotic regulation and chloride regulation in these forms. Smith (1959a) reports data for chloride regulation and for osmotic regulation in *Nereis limnicola.* His figures can be interpreted as indicating the onset of osmotic regulation at ambient concentrations of approximately 175 meq. Cl^-/l., although measurements were not made at concentrations just greater than this figure. Hence it is more accurate to say that this is the level at which internal osmotic concentration is maintained at ambient chlorosities between 30 and 150 meq. At 300 meq. there is no significant difference between internal and external osmotic concentrations. The data for chloride regulation indicate slightly higher regulated levels (approximately 210 meq.) with a similar ambiguity concerning the point at which regulation intervenes. Oglesby (unpublished) has kindly permitted the use of data concerning osmotic regulation in *Nereis succinea,* which are summarized in Figure 3. Freezing point depression of the ambient medium and of the coelomic fluid was measured. Regression lines from data at chlorosities greater than 265 meq. and data between 54 and 150 meq. were prepared. These lines intersect at approximately 225 meq. Cl^-/l. which may be taken as an estimate of the point of onset of osmotic regulation. It is apparent that the concentrations of the external medium at which osmotic regulation begins, at which chloride regulation commences, and at which there is a sharp modification of glycine accumulation correspond quite closely.

On the basis of this information, it can be suggested that the processes which underlie osmotic regulation are incompatible with the accumulation of amino acids from the ambient medium. In further support of this possibility, the following remarks may be offered. Essentially all the marine invertebrates examined (with the exception of marine arthropods), a total of approximately 40 genera in 10 phyla (Stephens and Schinske, 1961; Stephens, 1962a, 1962b, unpublished observations),

are capable of removing amino acids from dilute solution in sea water. As pointed out in the introduction to this work, none of 12 genera of soft-bodied fresh-water invertebrates in 6 phyla show this capacity. The ability of the nereids studied in the present report to take up glycine from solutions of galactose and sodium chloride at suitable osmotic concentrations is also suggestive.

With respect to the rate at which glycine is acquired by the two species of nereids, the rate exhibited by *Nereis succinea* is of the same order of magnitude as

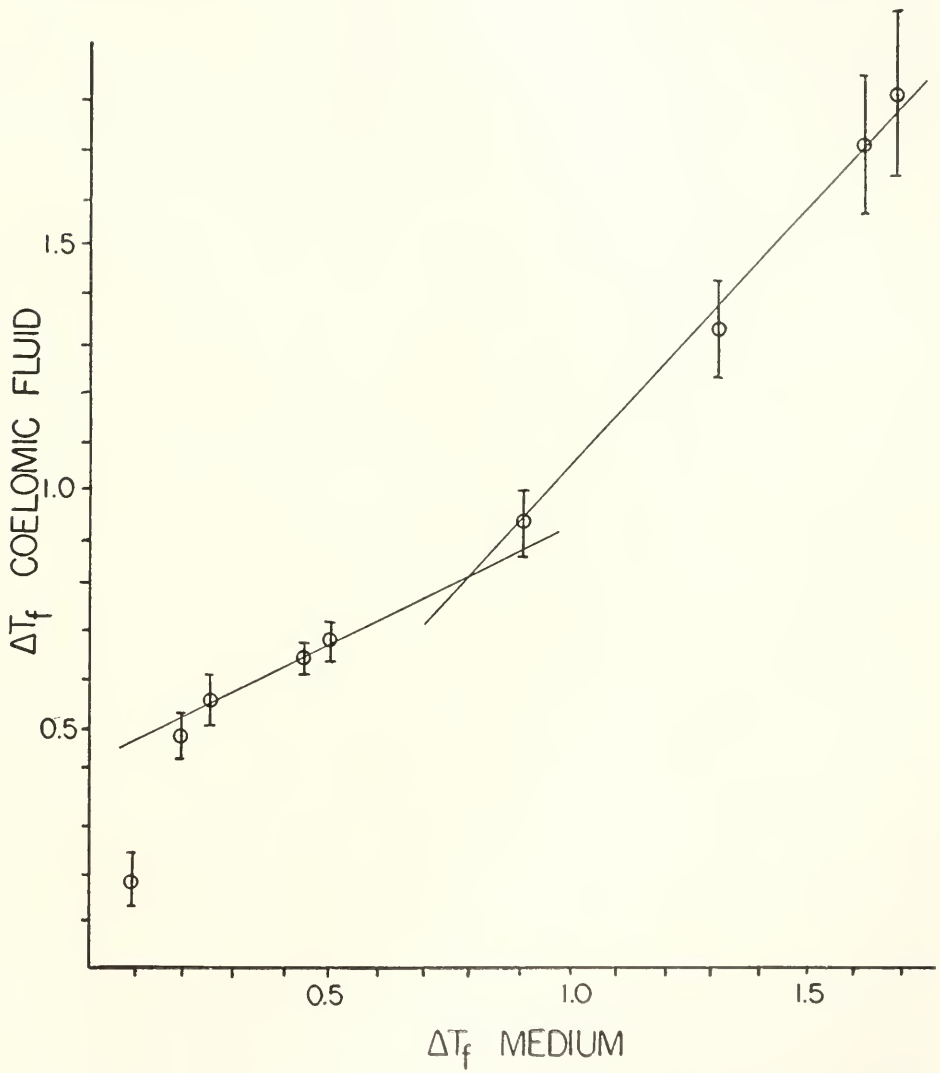

FIGURE 3. Freezing point depression of the coelomic fluid of *Nereis succinea* as a function of the freezing point depression of the medium. The bars represent standard deviations, and the lines are calculated regression lines (see text). Data furnished by L. C. Oglesby.

that exhibited by the marine worm, *Clymenella torquata* (Stephens, 1962b). Assuming that the metabolic rate of *Nereis succinea* is not grossly different from that of *Clymenella*, this implies that amounts of reduced carbon which are significant when compared with those required to support the oxygen consumption of the animal may be available by this pathway. This involves the further assumption that free amino acids are available in the environment. This appears to be the case in mud flats previously examined (Stephens, 1962c) and in other marine sediments (Belser, 1959, 1963) but has not been demonstrated in the brackish-water flats of San Francisco Bay where these animals were obtained.

By contrast, the rates of uptake exhibited by *Nereis limnicola* are an order of magnitude lower and it is difficult to imagine that this process could provide more than a small percentage of the carbon required by the animals. Furthermore, the accumulation system would only function in estuarine populations where salinity rose to the range which is permissive with respect to accumulation.

It is interesting to contrast the distribution and the ability of the two worms to regulate at extremely low salinities. *N. limnicola* tolerates exposure to distilled water and is found in a true fresh-water habitat. Figure 3 suggests that the ability of *N. succinea* to regulate osmotic concentration of the coelomic fluid may decline at very low ambient concentrations. The two species seem not to overlap in distribution. *N. succinea* replaces *N. limnicola* in brackish waters of higher salinity. The data concerning distribution and osmoregulatory ability are consistent with the position that osmoregulation and accumulation of amino acids are not compatible. They are also consistent with the speculation that the ability to accumulate amino acids may confer an adaptive advantage on organisms which inhabit marine or the more saline brackish-water habitats.

It is apparent that the process of adaptation to a change in salinity extends for a period of more than two weeks in *Nereis limnicola*. For this reason, the relation which is suggested by the data presented in Figure 1, relating uptake and ambient salinity between 200 and 543 meq. $Cl^-/l.$, should be considered tentative. In no case had the animals been maintained for sufficient time to consider that they had adapted to the sea water concentration at which the measurements were made. No evidence was found to suggest a prolonged adaptation period to salinity change in *N. succinea*. However, the critical procedure of studying worms which had been adapted to a low salinity and then returned to a high salinity was not carried out.

The observations reported here do not bear on the potential function of amino acids which the worms may acquire by this pathway. Presumably they would enter the free amino acid pool of the organisms and hence participate in a variety of oxidation and synthesis pathways. Some evidence has been presented that this is the case in other marine worms (Stephens, in press).

I wish to express my thanks to Prof. R. I. Smith of the Zoology Department at the University of California at Berkeley for his kindness in making space available to me in his laboratory for this work. I must also acknowledge his generous help and advice in collecting and maintaining the animals employed. I would also like to express my appreciation to L. C. Oglesby of the same department for permitting me to use his unpublished data concerning osmoregulation in *Nereis succinea*.

Summary

1. Both *Nereis limnicola* and *Nereis succinea* are capable of removing glycine from dilute solution in the surrounding medium at intermediate and high salinities. Uptake declines rapidly at sea water concentrations of chlorosity less than 200 meq./l.

2. The uptake is linear with time for periods of at least one hour, and appears to take place across the body wall without the necessary participation of the gut. Uptake is an exponential function of wet weight.

3. The relation between ambient concentration of glycine and rate of uptake suggests that an adsorptive step becomes limiting in the process of uptake at high concentrations of glycine. A plot of the reciprocal of uptake *versus* the reciprocal of ambient glycine concentration (Burk-Lineweaver plot) is linear and permits estimation of the apparent V_{max} and K_m for the process.

4. In *N. limnicola*, the adaptation of the physiological system mediating glycine uptake to a change in salinity is a process extending for more than 14 days.

5. Conditions which produce an increase in uptake of glycine at low ambient concentrations in *N. limnicola* also produce a significant increase in estimated maximum velocity and a decrease in the estimated K_m from a Burk-Lineweaver plot. This suggests that the increase reflects a change in the process which cannot be explained as merely an increase in the number of available sites for uptake, since this would not influence the K_m.

6. Both species are capable of removing glycine from solutions of galactose and of sodium chloride whose osmotic concentrations are approximately equal to that of sea water at 200 meq. Cl^-/l., although the rates are significantly below those observed in sea water.

7. The rate of uptake observed in *N. succinea* exceeds that in *N. limnicola* by an order of magnitude. This is correlated with the distribution of the latter species, which occurs at lower salinities, and with its greater osmoregulatory ability at low concentrations of the medium.

8. The salinity of the medium at which the process of glycine uptake declines agrees closely with estimates of the point of onset of osmoregulation and of chloride regulation in the two worms. This, together with the widespread ability of marine invertebrates to remove amino acids from solution and the failure to demonstrate this capacity for fresh-water forms, leads to the suggestion that the processes of glycine uptake and osmoregulation are incompatible.

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